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A CULTURED NEURON PROBE

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Abstract- **Work in progress toward the creation of a "cultured neuron probe" is described. This is a silicon structure similar to that for a multielectrode probe, but with embedded neurons which are intended to grow out and integrate with the neurons of a host nervous system into which the probe has been inserted. Each probe neuron is in close proximity to an electrode which is designed for extracellular stimation and recording from the probe neuron. In this way the probe neurons may provide a highly specific long-term communication link between external electronics and the host. Initial experiments to establish the feasibility of the concept will be done in rat hippocampus.**

I. INTRODUCTION

In order to create neural prostheses to help people better cope with such disabilities as paralysis, deafness, or blindness, there is a need for long-term connections to their nervous system. To date such communication has been established by using metal electrodes, which make non-specific contact to many cells in the neighborhood of the electrode tip. It would be desirable for communication with the central nervous system if there was specificity to a particular cell type. This could provide, for example, for recording control signals from the cortical pyramidal cells which drive spinal cord neurons. For visual stimulation, connections of the geniculo-cortical pathway could be mimicked. In addition, for chronic stimulation, it would be desirable to have neuron specificity to avoid change in effectiveness over time, which is observed with less selective electrical stimulation.

In recent years, researchers in neural transplantation have achieved long term survival of implanted neurons, and have observed cell-specific integration into the host nervous system [1]. Also, in recent years, silicon device microfabrication technology has evolved for the production of complex multielectrode probe structures for chronic implantation into the central nervous system [2]. This project involves a combination of these two developments. Silicon probe structures are being made which can hold captive neurons, in close proximity to an electrode. When the probe is implanted with neurons in it, it is hoped that axons and dendrites from these neurons will grow out and integrate with the host nervous system. The probe electrodes can then be used for two-way connection to the host by using the probe neurons as highly selective intermediaries.

II. PROJECT DESIGN

We have chosen the rat hippocampus as a trial target for the cultured neuron probe. Fig. 1 below shows a schematic view of the probe, along with a sketch indicating the placement of the probe in the pyramidal cell layer of the hippocampus of an adult rat.

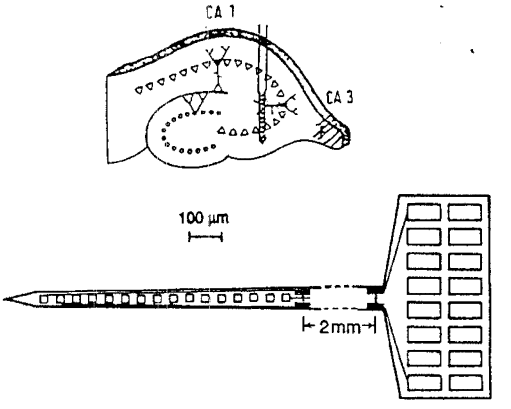

Fig. 1. Probe schematic and placement.

Neurons are dissected from day 18 rat embryos, and placed in the 16 probe wells shown schematically in the sketch. It is known from transplant studies that embryonic neurons are best suited for survival and outgrowth, and that they tend to make connections to what would be their normal targets. It is hoped therefore that probe neurons will integrate with the host hippocampal cells.

A neuron well structure is illustrated in Fig. 2 below. By using an anisotropic EDP (ethylene-diamine-pyrocatechol) etch, truncated-pyramid wells can be

formed under a patterned silicon dioxide grillwork. The central hole provides entry through which a dissociated neuron can be inserted, using a micropipette. Axons and dendrites growing out through the corner holes will then trap the neuron. Electrodes in the bottom of the wells are connected by insulated gold leads to pads at the probe handle, from which flexible leads can be brought to a skull plug.

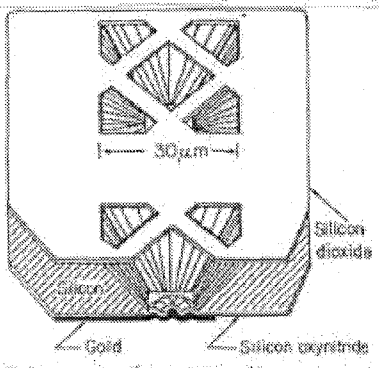

Fig. 2. Schematic drawing of a neuron well.

For the project to succeed, a number of issues must be addressed:

1. Probes must be fabricated, with processing and patterning on both sides of a 16 micron thick membrane.
2. Neurons must survive and grow out of wells.
3. The two-way electrical connection from probe electrode to neuron must be effective.
4. Neurons must survive and grow in the host.
5. The existence of probe-host communication must be established.

The group at Caltech has worked on *in vitro* studies and fabrication, to address isues 1-3, while the group at Rutgers has addressed issues 4 and 5.

III. FABRICATION

A thin membrane is formed from epi-wafers which incorporate a four micron thick heavily boron- doped layer under 16 microns of lightly doped (100) silicon. Using EDP, 5 x 9 mm "basins" are etched through the 500 micron thick silicon substrate to the boron etch-stop, to form thinned regions for probe formation. Using projection lithography, the grillwork is formed at the bottom of the basin, while electrodes and leads are formed on the opposite side. These connect to bond pads on full-thickness silicon surrounding the basin region, forming the probe handle. The scanning electron micrograph below, Fig. 3, shows the tip region of a completed probe.

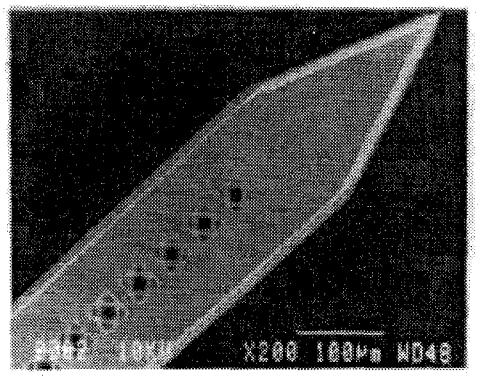

Fig. 3. The tip region of a completed probe.

In order to facilitate *in vitro* tests, "neurochips" are also made, which incorporate a 4 x 4 array of wells at the center of the basin, and have leads to bond pads at the periphery of a 1 cm square full-thickness chip which encompasses the basin [3].

IV. *IN VITRO* STUDIES

The well design shown in Fig. 2 was tested successfully for survival and outgraowth of rat sympathetic neurons. However, when hippocampal pyramidal cells were used it was found that trapping of the neuron was unsuccessful. After a short time the dendrites were seen to retract and the cell body climbed out of the corner hole along the axon (perhaps mimicking its behavior during development *in vivo*). In tests with wells containing exit holes as small as 1 x 4 microns, neurons roughly 15 microns in diameter deformed themselves so as to pass through the holes.

To successfully trap the hippocampal cells a new well design was made, and successfully fabricated. In this design the neuron processes grow out of the well through tunnels. This well design is shown at left in Fig. 4, viewed with Nomarski optics.

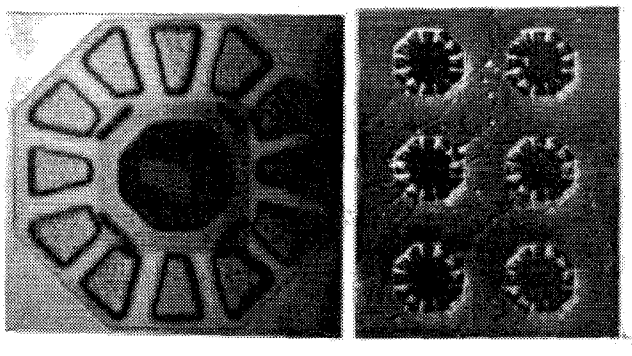

Fig. 4. Well with tunnels, and neuron outgrowth.

The octagonal shape shows the outline of a 0.5 micron thick silicon nitride canopy. The square gray area at the center defines the top of the well. A hole in the center of the nitride canopy provides for inserting a dissociated neuron. The four small slots at the corner of this area define the boundaries for the EDP etch, which undercuts the nitride to form the well. The 12 clear regions radiating outward from the center are tunnels, 0.5 microns high, 4 microns wide, and 15 microns long, through which neuron processes grow. The canopy is really not flat, but bends down 0.5 microns to anchor to the silicon substrate at the irregular gray areas between tunnels. The anchor regions and tunnels are formed by depositing nitride on patterned LPCVD low temperature silicon dioxide 0.5 microns thick, which is removed by etching with 48% HF to leave the final structure.

At the right in Fig. 4, part of the area of a neurochip with these wells is shown. Vigorous outgrowth of hippocampal cells through the tunnels is seen for neurons one day in culture.

A second set of *in vitro* studies has been made with "dummy probes" which have wells but not electrodes, in order to study outgrowth into cultured hippocampal slices. In order to observe the probe neurons and their processes a protocol for staining dissociated neurons with the membrane-bound stain DiI was developed. This staining allows observation of processes growing from the cells over a period of at least two weeks. It was observed that stained embryonic neurons grow vigorously into a slice from a probe placed on the surface. It was also discovered that exposing a probe loaded with neurons to dry air for even a few seconds causes cell damage from dehydration.

V. *IN VIVO* STUDIES

Initial studies have been directed toward determining whether probe neurons survive and grow in host adult rats. These studies have all been performed with dummy probes to look for anatomical evidence of outgrowth. The first method used was loading the probe with cholinergic septal neurons. These neurons and their processes can be identified after fixation with a dark brown cholinesterase stain. For probe tests, the adult rat was first subjected to lesioning of the septal-hippocampal pathway which provides normal cholinergic inputs. After these cholinergic axons died, the hippocampus, with no cholinergic inputs, was implanted with a probe carrying septal neurons.

The rats were perfused and fixed, and the brains sectioned in the vicinity of the probe, after survival times of one to three months. Techniques were established for sectioning in the probe area and parallel to the probe so as to be highly sensitive to outgrowth. In two cases, cholinergic staining was observed from processes which appeared to come from probe neurons. Fig. 5 below shows one example. Stained processes can be seen, growing several hundred microns out of the tip region of a probe.

Fig. 5. Stained processes growing out of a probe.

One reason for the low yield of septal outgrowth may have been the well design. Wells used at this time were found to have overhangs which inhibited outgrowth *in vitro*. Currently, studies are under way with DiI stained hippocampal pyramidal neurons in acute experiments in which probes are inserted and removed after short times. Poor survival, possibly due to dehydration, and overgrowth by a fibous layer, have been observed. Work is continuing to first achieve good short term survival before proceeding with longer term outgrowth experiments.

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